

BEHAVIOR AND ELECTRICAL ACTIVITY IN THE HYDROZOAN
PROBOSCIDACTYLA FLAVICIRRATA (BRANDT).
I. THE HYDROID COLONY

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During the past decade a number of workers have recorded electrical activity in hydroids while observing overt behavior. The gymnoblasts were *Cordylophora* (Josephson, 1961a, 1961b; Mackie, 1968), *Hydra* (Passano, 1962; Passano and McCullough, 1962, 1963, 1964, 1965; Rushforth, 1965a, 1965b, 1971; Rushforth and Hofman, 1972) and *Tubularia* (Josephson, 1962, 1965a, 1965b; Josephson and Mackie, 1965), and one calyptoblast, *Obelia* (Morin and Cooke, 1971).

Often these animals generate potentials which cannot be correlated with any obvious behavioral events, though occasionally it has been possible to designate one pulse type to a particular movement. Much of the activity has been described as spontaneous with pacemaker control.

Slow, precise postural movements are rarely accompanied by large potentials. If electrical activity can be recorded, at such times, the potentials are usually of short duration with a very low amplitude, often at the limit of the resolving power of the recording equipment (Mackie, 1968). Usually recording has taken place in a homogenous environment where sensory input is presumed to be at a low level.

The conditions under which recordings were taken from *Proboscidactyla flavicirrata* are in marked contrast to the above since *P. flavicirrata* is found only in association with sabellid polychaetes. Many of the recordings were made with the host sabellid in its tube and actively feeding, hence conditions could be regarded as particularly "noisy" (large sensory input) for the hydroid colony.

MATERIALS AND METHODS

P. flavicirrata (Brandt) is a gymnoblastic hydroid found as an obligate symbiont on the outside of the rim of sabellid tubes. The species appears to range throughout the northern coasts of the Pacific, at least as far north as the Queen Charlotte Islands and south to California.

The species found acting as hosts in this study were *Pseudopotamilla ocellata*, *Schizobranchia insignis* and possibly *Pseudopotamilla reniformis* (only 2 specimens of *P. reniformis* were found infested).

A brief description of the colony of *P. flavicirrata* is given here as it is so dissimilar from most other hydroid colonies. The colony consists of four types of individuals—gastrozooids, dactylozooids, gonozooids, and attached medusae (Fig. 1). Gastrozooids are normally confined to the rim of the tube with their mouths facing towards the axis of the tube. Each gastrozooid bears only two tentacles that arise on the side of the column which faces the host polychaete, just below

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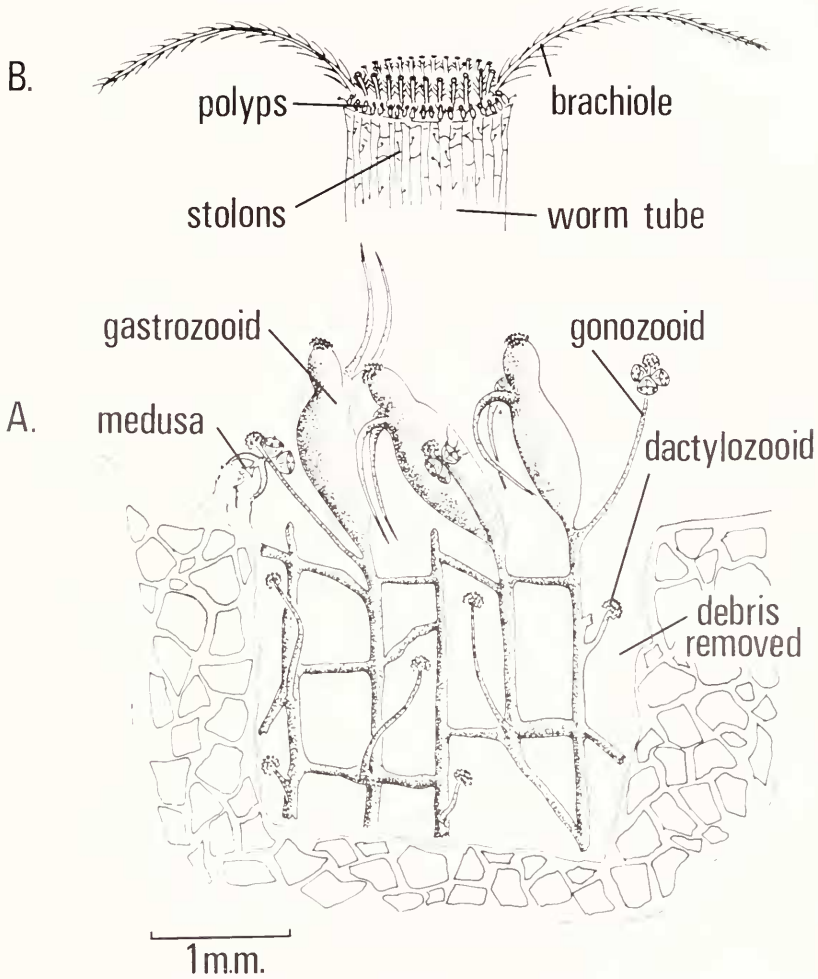


FIGURE 1. (A) Diagram of part of a colony of *P. flavicirrata* with the host worm removed, (B) appearance of the colony with the worm present. Only the proximal portions of most of the brachiolae of the worm are shown.

the "neck" of the hypostome. The apex of the hypostome carries a nematocyst pad (cnidothylacie) as do the terminal knobs of dactylozooids and gonozooids. Gonozooids arise close to the bases of the gastrozooids (unless the colony is rapidly advancing as the tube grows). Dactylozooids, which never bear medusa buds, are found further from the rim than gonozooids, usually on longitudinal stolons. In sexual colonies there are eight medusa buds to each gonozoid, each bud being at a different stage in development. In advancing colonies the stolons run parallel at the terminal end of the tube leaving a stationary mat of anastomosing stolons covering proximal portions of the tube. More usually transverse stolons are found at the bases of gastrozooids anastomosing with longitudinal stolons.

Infested sabellids were collected from floats and wharves at the southern end of Vancouver Island and San Juan Island, then transferred to either a non-recirculated natural seawater system (Friday Harbor Laboratories) or a recirculated system (University of Victoria).

In the former system both the hydroids and their hosts obtained an adequate food supply whereas in the latter system the hydroid colonies eventually regressed after a few weeks. Water temperatures ranged from 9 to 13° C and no strict photoperiod was maintained.

Electrical activity was recorded using polyethylene tubing drawn out to a fine tip (20–100 μm), with a length of platinum or Ag/AgCl wire acting as the conductor and sea water as the electrolyte. The electrodes were connected to a syringe so that they could be sucked onto the ectoderm of the hydroid. Ag/AgCl suction electrodes were used to deliver shocks.

The pre-amplifiers of the Grass Model 7 polygraph were used to drive the oscilloscope (Tektronix 502A). Separate capacity-coupled pre-amplifiers were sometimes used with a Gilson Model M5P polygraph. Normally the polygraphs were operated with a differential input, though ground reference recordings were also found necessary. Oscilloscope displays were photographed with a 5-inch Polaroid camera.

Sea water in the recording dish was cooled (11–14° C) by water passing through a glass coil partially sunk into the wax-base of the recording dish.

RESULTS

Behavioral observations

Feeding. The host sabellids feed continuously throughout the day, unless disturbed, when the fan is withdrawn for several minutes. While the fan is erect all three host species continuously create centripetally directed water currents passing through the brachioles of the fan. This current is maintained, presumably for respiratory purposes, even when suspended food material may be scarce. In contrast, gastrozooids feed only when there is abundant food.

A typical microphagous feeding cycle for a single gastrozooid is as follows: (1) The tentacles of the gastrozooid are positioned along the long axis of the food grooves of neighboring brachioles of the sabellid (In most colonies the density of gastrozooids is such as to position individuals between brachioles.)

(2) Both tentacles are extended by relaxation of the longitudinal muscles and elasticity in the mesogloea and possibly the vacuolated endodermal cells (circular muscles are absent (Spencer, 1971)). Tentacles relax by up to 25 times their contracted length.

(3) The tentacles extend down the food grooves towards the oral region of the worm, on occasions they may reach as far as the palps.

(4) The gastrozooids remain in this position for times varying between 10 seconds and several minutes.

(5) Then the tentacles are withdrawn rapidly by either a single contraction or a flurry of contractions of the longitudinal muscle, with flexion in an oral direction. In addition an assymetrical contraction of the longitudinal muscles of the column often occurs, causing the gastrozooid to bend away from the rim.

(6) Usually the tentacles are coated with a mixture of mucus and food particles, and at such times the tentacles are wiped through the mouth singly or together. The mouth gapes, only if food is covering the tentacles (proline at 10^{-5} M induces gaping). The oral hood engulfs the proximal portion of the tentacle first, then the tentacle, in its contracted state, is wiped through the mouth. This stage can take as long as 1 minute.

(7) If food is not present on the tentacles then they are not swallowed and frequently return to Stage 1.

(8) The mouth is closed after both tentacles have been wiped and a slow peristaltic wave passed down the neck pushing food into the gut.

Most of the food gathered in this way consist of unicellular algae and some early larval crustaceans. Nematocysts are not used at such times. In addition to the microphagous feeding cycle described above gastrozooids are capable of capturing and ingesting large prey (*Artemia* nauplii, harpacticoid copepods, nematodes and eggs of the host worm) by using nematocysts. This macrophagous feeding cycle is similar to that described for *Hydra* (Josephson, 1965c).

Behavior while fan is retracted. Immediately following fan withdrawal by the sabellid, feeding often recommences at Stage 5 or 6 for one cycle. When the feeding cycle is completed the tentacles are not extended as would be the case if the fan were erect. However, gastrozooids continue to make abortive feeding movements as in Stage 5 continuously until the fan is erected once more. The tentacles are never wiped through the mouth.

The frequency of tentacle contraction and column bending in any one individual decreases from a mean value of 16/ min just after fan withdrawal to about 1 every 15 min if the fan remains withdrawn for several hours. Behavior of gonozooids, dactylozooids and medusa buds is not influenced by the presence or absence of the fan.

Behavior while fan is erect. Fan erection is far slower than fan withdrawal, often taking 30 sec to become fully expanded, whereas withdrawal takes place in less than 1 sec. As the fan slowly emerges from the tube the gastrozooids may rear backwards in response to a wave of colonial retraction (see below), but more usually they relax the tentacles allowing them to trail over the pinnules as they emerge. This is not a prelude to feeding as might be supposed, as tentacles are not swallowed. When the fan is erect gastrozooids go through the feeding cycle as already described.

Protective retraction of the colony and associated responses in the attached medusae. Colonial retraction involves all four types of individuals in the colony and is seen under a variety of circumstances. Withdrawal of the fan by the worm or animals clambering over the colony usually causes colonial retraction. Occasionally colonial retraction occurs when there is no obvious exogenous stimulation.

Basically the response comprises a wave of excitation spreading out from the point of stimulation, involving contraction of all the individuals in the colony. Any portion of the entire surface of the colony, except for a few distant dactylozooids and stolons, can act as a receptor site and set off a wave of contraction. Gastrozooids and tracts of stolon near the rim have lower thresholds than other regions of the colony. Tapping the tube, stolons, gastrozooids or gonozooids with a probe can set off a wave of colonial retraction. As a wave of excitation reaches each individual

TABLE I
Responses of attached medusae to colonial excitation

Size group (μm diam.)	State of tentacles (inside or outside bell cavity)	Response to excitation	Normal activity
0-279	Inside	None*	None*
250-420	Inside	Swim	None
400-490	Inside or outside	Swim or crumple	Swim
490-630	Outside	Crumple	Swimming
630-850	Outside	None	Swimming or crumple

* A response may not have been seen because the young medusa forms a virtually incompressible body at this stage.

the following muscular responses are seen: (1) Gastrozooids, tentacles are contracted synchronously, together with a contraction of the column. (Unlike feeding there is always shortening of the column.) (2) Dactylozooids and gonozooids, muscular activity consists of slight bending and shortening of the terminal quarter of the individual. (3) Medusae, the type of muscular response seen is dependent on the size of the medusa (hence its developmental stage) and is presented in Table I. This table has been condensed from observations made on eight medusae in each size class. The position of the four tentacles is used as an indicator of the developmental stage reached by the medusae. The word "swim" refers to one swimming contraction of the circular muscles. The word "crumple" refers to a contraction of the radial muscles which causes the margin of the jellyfish to be drawn up into the subumbrellar cavity (Hyman, 1940).

Frequently a wave of colonial retraction precedes fan withdrawal, thus preventing feeding gastrozooids from becoming damaged as the brachioles scrape down against the inside of the tube. The precise form of the stimulus initiating colonial retraction, in this case, is difficult to determine. However, if the chaetae of the sabellid are removed from the anterior segment of the worm colonial retraction is only rarely seen preceding fan withdrawal. Colonial retraction then occurs during fan withdrawal. Scraping of the setae and uncinii on the inside of the tube as the worm retreats would seem to be a stimulus capable of setting off a colonial retraction.

Electrical activity

Since gastrozooids dedifferentiate when the worm is removed from the tube it is almost impossible to obtain colonies that have been starved for extended periods. Thus all the recordings are taken from colonies that are, at the worst, poorly fed but certainly not starved. Table II summarizes some of the parameters of the pulse types recorded.

Tentacle contraction pulses (TCP's). Tentacle contraction pulses can be recorded from any part of the surface of the tentacles and column of the gastrozooid. They are the electrical accompaniment to tentacle contraction seen during feeding and abortive feeding.

The way in which tentacle contraction pulses can be stimulated artificially fall into three groups: mechanical, electrical and chemical stimulation. Prodding a

TABLE II
Parameters of the pulse-types

Pulse type	Initial polarity	Duration (msec)	Conduction velocity (cm. sec.)	Amplitude (mV)	
				+	-
Tentacle Contraction Pulses $N = 950$					
(i) Active tentacle (n = 358)	+	100-440 $\bar{x} = 206$	n.a.	1.0 -3.9 $\bar{x} = 2.6$	0.6 -2.5 $\bar{x} = 1.2$
(ii) Passive tentacle (n = 272)	-	80-160 $\bar{x} = 123$	n.a.	0.07-0.8 $\bar{x} = 0.25$	0.4 -1.9 $\bar{x} = 1.3$
(iii) Column (n = 320)	-	40-230 $\bar{x} = 143$	3-7 (14° C)	0.04-0.8 $\bar{x} = 0.39$	0.24-2.8 $\bar{x} = 1.48$
Column Contraction Pulses $N = 54$	+	100-420 $\bar{x} = 203$	n.a.	0.5-2.9 $\bar{x} = 2.3$	0.01-1.8 $\bar{x} = 0.5$
Colonial Pulses, $N = 782$	-	35-130 $\bar{x} = 78$	0.9-9.0 (14° C)	0.23-7.2 $\bar{x} = 1.4$	0.12-3.2 $\bar{x} = 1.3$

tentacle will elicit a single TCP or a burst of such pulses (Fig. 2A), whereas stretching a tentacle causes rhythmical firing (Fig. 2B), the frequency of discharge increasing as the stretching force increases. Single shocks of 1 msec duration delivered to a tentacle evoke a single TCP and never cause multiple firing of TCP's (whatever the stimulus strength).

The only amino/imino acid to cause a noticeable increase in the rate of TCP's was proline (Fig. 3A), which also causes mouth gaping.

Reduced glutathione (a tripeptide) at a final concentration of 10^{-5} M does not elicit tentacle contraction or mouth gaping. However the frequency and duration of TCP's after crustacean tissue fluids are added far exceed those seen after the addition of proline alone (Fig. 3B).

In an intact colony, with the polychaete in its tube, the rate of TCP firing in a gastrozoid is directly correlated to the activity of the worm. While the fan is

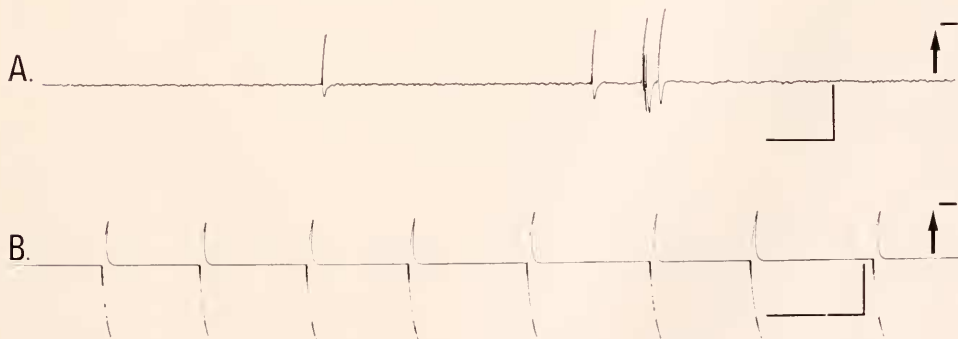


FIGURE 2. Tentacle contraction pulses recorded from two gastrozoids, (A) a single TCP and burst of TCP's recorded from the column of a gastrozoid after prodding a tentacle. The vertical bar represents 1 mV and the horizontal bar 5 sec; (B) rhythmical firing of TCP's when a tentacle is stretched by sucking it into the recording electrode. The vertical bar represents 1 mV and the horizontal bar, 5 sec.

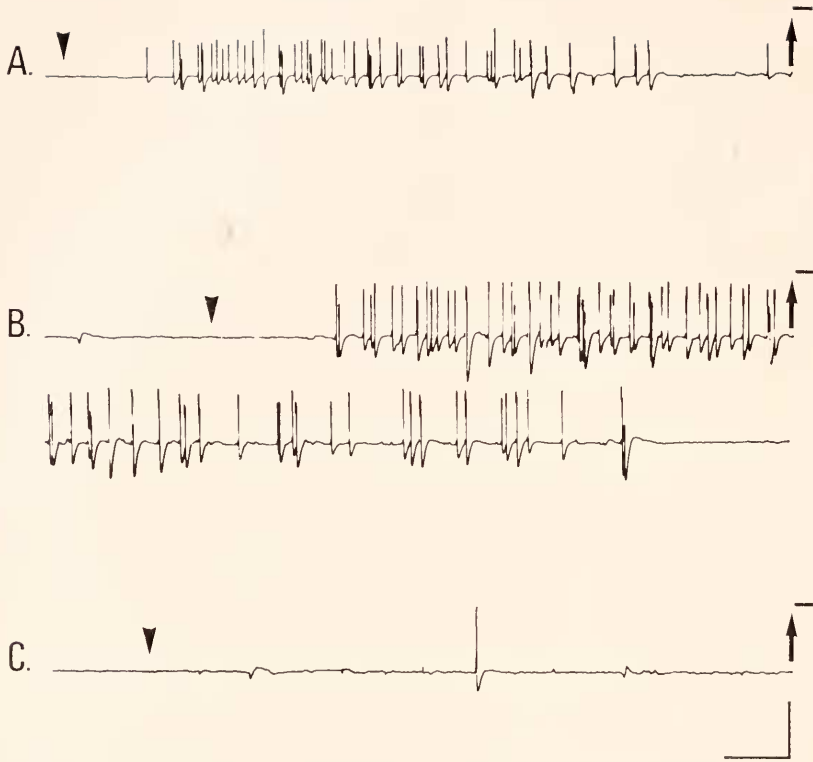


FIGURE 3. Chemical stimulation of tentacle contraction pulses; (A) a burst of TCP's recorded from the column after addition of proline at a final concentration of $10^{-6}M$ at the arrow; (B) a far longer burst of TCP's after addition of shrimp tissue fluid, also recorded from the column; (C) a single TCP after addition of an equal volume of sea-water at the arrow. Vertical bars represent 1 mV and horizontal bars 5 sec.

erect the rate of TCP firing is fairly constant, 1 pulse or burst every 40 seconds being a typical figure (corresponds to the average duration of a feeding cycle).

If particulate food is scarce the frequency is often higher than this, perhaps 1 pulse every 25 seconds (corresponds to the average duration of an abortive feeding cycle).

Immediately following withdrawal of the fan the frequency of TCP's is high, reaching a rate of 1 pulse every 8 seconds in any one individual. This frequency decreases as the period of fan withdrawal increases until the firing frequency may be as low as 1 pulse every 15 minutes. The rate of TCP firing may increase momentarily during fan erection.

The wave-form of a tentacle contraction pulse differs greatly depending on the recording position. If the recording electrode is positioned on the tentacle which is contracting, then the pulse appears as a biphasic event with the positive-going component being most noticeable (Fig. 4). When the electrode is placed on the other passive tentacle forming a pair then the event appears as a negative-going potential with a small positive overshoot (Fig. 4). Similarly, a TCP recorded

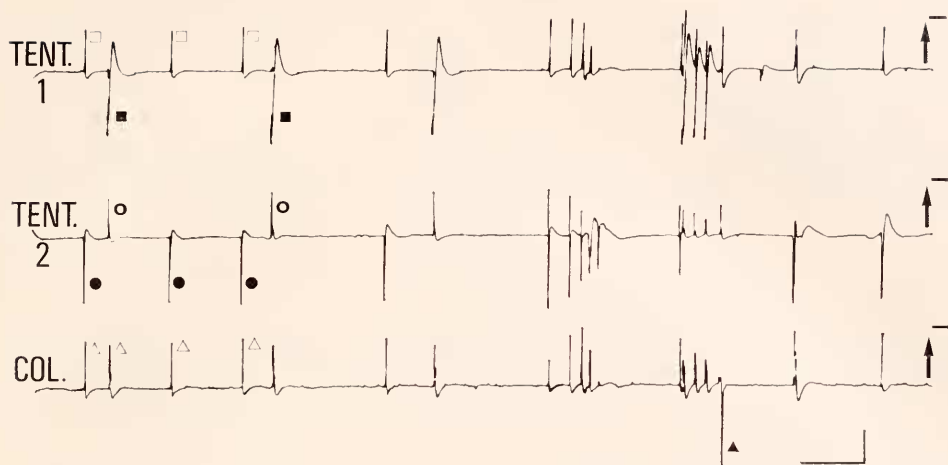


FIGURE 4. One column contraction pulse (CCP) occurring with a number of tentacle contraction pulses (TCP's) recorded from one gastrozoid. One electrode is attached to each tentacle and a third attached to the column. Symbols are: Filled squares, TCP of tentacle 1; filled circles, TCP of tentacle 2; open circles and squares, TCP recorded from passive tentacle; open triangles, TCP recorded from the gastrozoid; and filled triangles, CCP of the gastrozoid. The horizontal bar represents 5 sec and the vertical bar 1 mV.

from the column of a gastrozoid appears as a negative-going potential with a small positive overshoot (Fig. 4).

Conduction velocities of TCP's range from 7.8 cm/sec in the tentacles to 3 cm/sec on the abaxial surface of the gastrozoid measured from individuals partially relaxed, with the tentacles as long as the column (all at 15° C conducted in a distal direction).

Column contraction pulses (CCP's). Column contraction pulses can only be recorded from the column of the gastrozoid. If they are conducted up into the tentacles the amplitude of these pulses must be less than 5 μ V. Each CCP corresponds to a contraction of the column longitudinal muscles with fibrils on the abaxial side shortening more than those on the axial side of the column. The frequency of TCP's is always less than that of CCP's.

CCP's can either appear alone or with TCP firing of both tentacles. Conduction velocities of CCP's have not been measured since it is difficult to stimulate CCP's electrically without generating other pulse types, and not enough spontaneous CCP's were recorded to give a statistically reliable value. Figure 4 shows a column contraction pulse appearing in a burst of TCP activity.

Colonial pulses (CP's). Colonial pulses can be recorded by suction electrodes attached to any part of the external surface of the colony (the medusa just prior to release shows no electrical connectivity to the parent colony).

The passage of each colonial pulse through an individual causes protective retraction of that individual as described in the section on behavior. Single monophasic square-wave shocks of 500 μ sec, or longer, duration delivered at any position on the surface of the colony will generate a colonial pulse. Multiple firing is rarely seen.



FIGURE 5. Superimposed oscilloscope traces of repetitively stimulated colonial pulses (1 pulse every 2 sec) recorded from the bases of two widely separated gastrozooids. Six shocks were delivered on a stolon close to electrode 1 which recorded six colonial pulses. Each successive colonial pulse was conducted at a lower velocity than its predecessor with the last pulse failing to arrive at electrode 2. The horizontal bar represents 50 msec and the vertical bar 1 mV.

There is evidence that the phenomenon of facilitation does exist in the colonial pulse conducting system, but such evidence is not consistent. For example, in one colony the first shock delivered to the colony at the base of a gastrozoid may fail to elicit a CP, but the second shock delivered 2 sec later (at the same voltage) does set off a colonial pulse. Two hours later, with the same preparation, the first shock gives a colonial pulse.

Usually a colonial pulse generated by the first shock is conducted throughout the colony. Occasionally the first CP is not conducted to the furthest part of the colony, whereas the second and third CP's stimulated within a few seconds do reach previously unexcited areas. After perhaps twenty pulses, the distance through which each pulse conducts begins to decrease, until suddenly the whole colony becomes refractory to further excitation.

Since the stolon network is so complex, with numerous cross connections, the distance a CP must travel from the stimulating site to the recording electrode is difficult to measure. The number of alternate routes runs into many thousands.

It must be remembered that a CP travels as a wave of excitation eventually affecting all parts of the colony, thus many stolons are transmitting the same colonial pulse simultaneously.

Conduction velocities of CP's measured from long tracts of stolon using two recording electrodes give a mean value of 1.9 cm/sec (13° C, $N = 45$) with a minimum value of 0.9 cm/sec (13° C). In comparison measurements from short lengths of stolon without any cross connections or sharp bends give a mean value of 7.3 cm/sec (13° C, $N = 38$) with a maximum value of 9.0 cm/sec (13° C). These differences are presumably due to an inability to correctly measure the conduction distance in long tracts, they being considerably underestimated.

All parts of the stolon network are unpolarized as regards both conduction

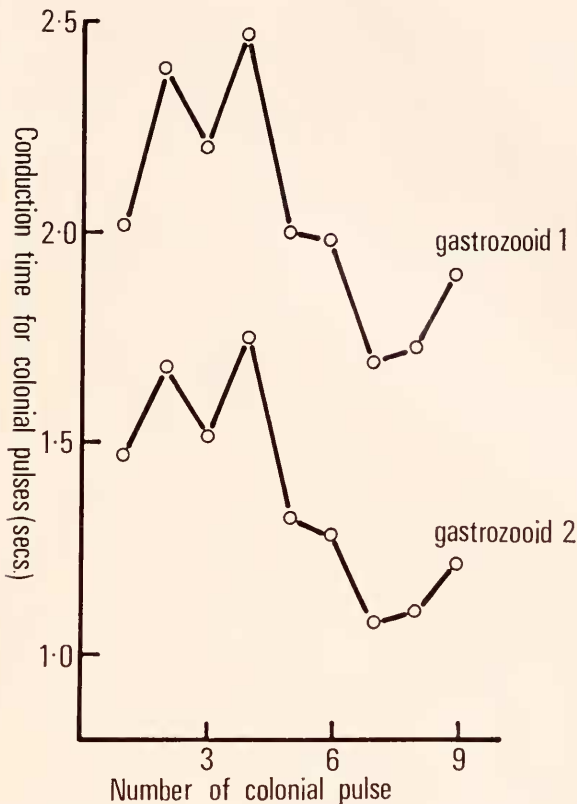


FIGURE 6. Graph to show that fatigue and recovery from fatigue of the colonial pulse system affects all parts of the colony equally. Nine shocks were given randomly to the colony over a period of one minute at a distant stimulating electrode. The time taken for each colonial pulse to reach two gastrozooids is shown.

velocity and facilitation. Conduction velocities are not significantly higher in large diameter stolons.

The conducting system for colonial pulses is particularly labile. At a constant stimulation rate of 1 shock/sec the system gives one CP for each shock for between 5 and 60 shocks, then follow periods of firing to alternate shocks, every third, fourth, fifth and sixth shock until the system no longer conducts. This effect can be brought about more rapidly by increasing the rate of stimulation. If a colony is stimulated with repetitive shocks having a frequency greater than 1 every 5 sec, then the conduction velocity of each colonial pulse decreases noticeably until the conducting system becomes inexcitable (Fig. 5). Figure 6 shows that the rate of fatiguing and recovery from fatigue affects all parts of the colony equally. As might be expected the threshold for generating a colonial pulse increases as the preparation fatigues.

Colonial pulses continue to be propagated after the colony has been immersed in a solution containing 1 part of sea-water to 1 part of isotonic magnesium chloride for three hours.

DISCUSSION

It is not surprising that the earliest accounts of the hydroid phase of *Proboscidactyla* include graphic description of the tentacle contraction and column bending at Stage 5 of the feeding cycle, since these movements are immediately obvious (Gosse, 1857; Hincks, 1872). However these authors only hint at the functional significance of such movements.

Even the most recent account of feeding (Hand and Hendrickson, 1950) fails to mention tentacle swallowing. These authors believe that back-bending positions the tentacles at right angles to the incoming water current, enabling gastrozooids to catch particles in this stream. Unfortunately, they do not describe how the food material is ingested.

Why does the genus *Proboscidactyla* have only two tentacles? If *Proboscidactyla* is not essentially a predatory hydroid then only two possibilities remain. Either it could be feeding on suspended, non-motile food as it is carried across the branchial crown of the sabellid, or it could be taking this food after it has been concentrated in the food-grooves of the host sabellid. The first possibility seems unlikely since it is an accepted principle that suspension feeders present a large collecting surface to the food-carrying current. Indeed the observations made in this study support the proposition that *Proboscidactyla* could be treated as a deposit-feeder despite the parasitic character.

Proline, rather than reduced glutathione, may well be the most important substance controlling feeding in the marine and brackish-water gymnoblastic hydroids, since *Cordylophora* and *Pennaria* as well as *Proboscidactyla* give a feeding response to proline, but not to reduced glutathione (Fulton, 1963; Lenhoff, Muscatine and Davis, 1968).

There is no doubt that *P. flavicirrata* consumes a considerable quantity of plant material (which may or may not be utilized). This is a habit not frequently met with in the Cnidaria.

A number of colonial hydroids show propagated retraction of polyps when mechanically stimulated (Wright, 1856; Zoja, 1891; Josephson, 1961a). The muscular responses seen in *P. flavicirrata* most resemble those seen in *Hydractinia* (Josephson, 1961a). Feeding polyps in both species contract while the defensive individuals (spiral zooids and dactylozooids) wave about.

The responses of attached medusae to colonial excitation have not been previously described. Varying responses of medusae to colonial excitation is predictable if one considers the tissue connections of the medusa-bud with the colony. Size group 400–490 μm has both an ectodermal and endodermal connection with the colony and responds to a colonial pulse by either swimming or crumpling. In medusae of 490–630 μm diameter the endoderm has been nipped off and the medusae can only respond by crumpling. Just prior to release only a mesogloea neck remains and neither swimming or crumpling can be elicited by a colonial pulse. From what is known of swimming and crumpling in the mature medusa and the conducting routes responsible for such behavior (Spencer, 1971), it seems probable that the ectoderm is conducting the colonial pulse so as to cause crumpling whilst a colonial pulse conducted in the endoderm causes swimming. Where both ectoderm- and endodermal connections are present between the gonozooid and medusa

a CP can cause either swimming or crumpling, with swimming being the common response in younger attached medusae.

Most of the hydroid species so far used for electrophysiological investigations tend to become electrically silent when starved (Passano and McCullough, 1964; Mackie, cited by Josephson, 1965b; Mackie, 1968). From what is known of the biology of *P. flavicirrata* it is very unlikely that colonies ever experience extended periods of starvation. Poorly fed colonies can be recognized in the laboratory, but at no time were obvious differences in the electrical activity of such colonies noticed, as compared to well-fed colonies.

Since every TCP is accompanied by muscular contraction, and the frequency of TCP's is controlled, to a large extent, by exogenous events then the system generating these potentials cannot be called pacemaker-like by the definitions that have come to be accepted by coelenterate electrophysiologists. This does not deny the possibility of some underlying pacemaker control of TCP firing. Judged from the very different wave-form of TCP's recorded from regions where muscle is contracting and passive neighboring areas, TCP's are likely to be compounded from epithelial excitation and electrical events associated with excitation-contraction (muscle potentials). Thus a TCP recorded from a tentacle which does not contract would be an epithelial potential with perhaps some contribution from underlying nerve elements.

Comparisons can be made, if somewhat cautiously, between tentacle contraction pulses and similar potentials in other hydroids. Where rhythmical potentials, associated with muscular activity in tentacles, have been recorded in other hydroids a pacemaker system or systems is always tied in with these rhythmical potentials. For example, in *Tubularia* tentacle potentials accompany oral flexion of tentacles with no synchrony between tentacles except during hydranth pulse bursts (Josephson and Mackie, 1965). In *Hydra* column contraction pulses (also involving tentacle contraction) occur in bursts with a predictable rhythmicity that is dependent on such factors as light intensity, feeding, mechanical stimulation and a pacemaker system, the rhythmic potentials (Passano, 1962; Passano and McCullough, 1962, 1964).

The observation that TCP's can be set off by stretching a tentacle suggest that either the epithelio-muscular cells can be excited by stretching or that the tentacles contain stretch receptors. This has already been suggested for the chondrophoran *Porpita* by Mackie (1959).

Under conditions where it can be expected that TCP's will be generated by excitation of sensory cells, TCP's appear as bursts; for example after mechanical and chemical stimulation. If, on the other hand, the intervention of sensory cells is bypassed then single TCP's are generated after giving above threshold shocks. There is no evidence of neuro-muscular facilitation in the TCP conducting system, one supra-threshold shock always causes contraction. Even in anemones where neuro-muscular facilitation has been clearly demonstrated (Pantin, 1935a, 1935d) tentacles respond earlier in a stimulus burst than does the sphincter (Josephson, 1966).

In no other hydroid has an electrical event, been recorded with so much control over its frequency coming from outside the animal. This extrinsic control appears to dominate any underlying endogenous rhythm. It is only during periods when

the fan is retracted that the TCP frequency may settle down to a fairly constant level with no obvious exogenous stimulation. Much of the control of TCP firing therefore comes from the host worm which is to be expected in a relationship where the hydroid is a dependent. It is of great advantage to the colony that its behavior be closely related with the host's, particularly when both are feeding.

The frequency of column contraction pulses is controlled by the same factors as TCP's except that proline and crustacean tissue fluid does not increase the rate of firing. Indeed the recordings of TCP bursts after addition of feeding activators never include CCP's. This could indicate that the receptors are located in the tentacles and/or that excitation from the chemoreceptors is not conducted to the column musculature. Rushforth, Krohn and Brown (1964) have shown that the receptors of G.S.H. in *Hydra* predominate in the tentacles.

The occurrence of TCP's in both tentacles and with CCP's is rarely synchronized, except of course after colonial pulse excitation. It seems that simultaneous contraction of the longitudinal musculature in both tentacles, perhaps with column contraction, is a chance happening. These three muscle systems must either be controlled by separate pacemakers or, as seems more likely, by separate sensory systems. What is also surprising is that excitation of one muscle system does not cause contraction in neighboring systems even though large potentials are being conducted by cells in these regions, for example TCP's recorded from a passive tentacle.

For many years now coelenterate conduction systems have been classified into two broad categories, through-conducting systems and facilitating systems (Pantin, 1935a, 1935b, 1935c, 1935d; Josephson, 1965b, 1965c). Any attempt to fit the colonial pulse system of *P. flavicirrata* into either of these categories fails. In most preparations conduction of colonial pulses meets the requirements of a through-conducting system, but other preparations show incremental properties with successive pulses travelling greater distances through the colony. Similarly the system can also be decremental in respect to conduction distances, velocities and its refractoriness with fatigue.

Within the Hydroidea the Hydractinidae possess colonial co-ordinating systems that most closely resemble that found in *P. flavicirrata* (Josephson, 1961a). Josephson's studies show that gymnoblastic hydroids not belonging to these groups do not possess all-or-none conducting systems that give colony wide excitation to the first shock. It should be noted that in *Hydractinia echinata* the colonial system can facilitate, but this is unusual. Within the corals only the perforate madreporaria give colonial retraction to the first shock with additional shocks facilitating the spread of excitation, except *Acropora* (Horridge, 1957).

Of the facilitating types of systems that found in *Cordylophora lacustris* has been most carefully studied (Josephson, 1961b). Electrical stimulation of the stolon of a *Cordylophora* colony causes bursting of pulses, the number of pulses and their amplitude facilitating with repetitive stimulation and the intensity of the shock. The greater the number of pulses the greater and faster is contraction of the polyp nearest the recording electrode. Also the number of pulses in each burst determines the distance that the excitation spreads through the colony. At no time did *Proboscidactyla* give multiple colonial pulses to a shock. One other colonial cnidarian should be considered, the siphonophore *Nanomia cara*. This animal is capable of a defensive motor pattern that causes stem contraction and

synchronized, reserved swimming in the nectophores (Mackie, 1964). The excitation is conducted by ectodermal tissues, at least in parts of the colony, and can be elicited by a single shock. *Nanomia*, however, has evolved a more sophisticated conducting system than has been found in any other cnidarian which involves the use of gigantic syncytial cells in the stem (Mackie, unpublished observations) enabling high conduction velocities upto 100 cm/sec (14° C) (Mackie, 1964).

It seems likely that neurons and muscular elements are absent from the stolons of *P. flavicirrata* (Spencer, 1971) and thus the spread of colonial pulses through the stolonal network is a purely epithelial event. Within the individuals of the colony however nerve and muscle cells may contribute to the spread of excitation. Observations on the responses of attached medusae to colonial pulses as they mature and are finally released indicate that both the ectoderm and endoderm are involved in conducting colonial pulses within the stolons and gastrozooids. The fact that CP's continue to be propagated after exposure to excess Mg^{++} also suggests that conduction in this system is non-nervous since magnesium ions are known to have an inhibitory effect on the appearance of miniature potentials at the post-synaptic side of chemical junctions (Katz, 1962). Other hydrozoan (*Sarsia*, *Euphysa*, *Nanomia*) epithelial conducting systems are capable of functioning in the presence of excess Mg^{++} while supposed neuronal pacemakers and neuromuscular events are blocked (Mackie, 1964; Mackie and Passano, 1968). However this does not mean that susceptibility to magnesium anaesthesia can be used to unequivocally distinguish nervous from non-nervous conducting systems, as Mackie and Passano (1968) were careful to point out.

That recent studies of epithelial conducting systems in the cnidaria have not revealed the occurrence of velocity changes that depend on the rate of stimulation is remarkable. McFarlane (1969) may have recorded similar events in *Calliactis parasitica*. He showed that in the column of *Calliactis* there is a slow conducting system (SS_1) that is probably epithelial. Using just one recording electrode he demonstrated that the time taken for each potential of the SS_1 to reach the recording electrode increases with repetitive stimulation if the stimulating frequency is between one shock/3 sec and one shock/20 sec. At these rates the system eventually fails to conduct. McFarlane interprets these results as being due to an increase in the delay taken to generate a pulse at the stimulating site. Unfortunately he did not test the possibility that the increased delay in arrival of each pulse at the recording electrode may be mostly due to a decrease in conduction velocity rather than increased generation time.

A similar fatigue phenomenon to that seen in *Proboscidactyla* has been reported recently in chick cardiac muscle where repetitive stimulation of slow conducting regions caused a 2:1 block at 4 beats/sec which was preceded by a cyclic, progressive slowing of conduction (Lieberman, Kootsey, Johnson and Sawanobori, 1973). A full discussion of the likely mechanisms of such fatiguing in the colonial pulse system will be included with an analysis of a similar epithelial event described in a following paper on the medusa of *Proboscidactyla flavicirrata*.

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SUMMARY

1. *P. flavicirrata* feeds on material collected from the brachiolar food-grooves of the host sabellid worm.

2. The feeding cycle involves wiping the tentacles through the mouth. An abortive feeding cycle can also be seen when the tentacles are not wiped through the mouth.

3. The frequency of the feeding cycle is in part controlled by activities of the host worm.

4. Tentacle contraction pulses and column contraction pulses are the electrical accompaniment to feeding and abortive feeding. Their properties are described.

5. Colonial pulses, conducted in part by epithelial cells at velocities between 1.9 and 7.3 cm/sec (13° C), cause protective retraction of all the individuals in the colony. The varying response of medusa buds as they develop is described. The colonial pulse conducting system fatigues rapidly after repetitive stimulation.

6. Colonial pulses continue to propagate after exposure to excess Mg^{++} for 3 hr.

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